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AD NUMBER

**AD821460**

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**D/A ltr, 29 Sep 1971**

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**AD821460**

**AD**

**TECHNICAL MANUSCRIPT 408**

**AN IMPROVED METHOD  
FOR PARTIAL PURIFICATION  
OF THE ORGANISM OF PSITTACOSIS  
GROWN IN CHICKEN EMBRYO YOLK SAC**

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AD821460  
RECORDED 8/24/67  
AUGUST 24 1967  
RECORDED

**AUGUST 1967**

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DEPARTMENT OF THE ARMY  
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TECHNICAL MANUSCRIPT 408

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OF THE ORGANISM OF PSITTACOSIS GROWN IN  
CHICKEN EMBRYO YOLK SAC

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Project 1B533001D426

August 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

AN IMPROVED METHOD FOR PARTIAL PURIFICATION OF THE ORGANISM  
OF PSITTACOSIS GROWN IN CHICKEN EMBRYO YOLK SAC

ABSTRACT

A simple procedure is described for obtaining the organism of psittacosis employing successive extractions with (i) calcium chloride, sodium dextran sulfate, and sucrose (CC-DS-S) and (ii) Freon-114. Compared with the CC-DS-S or Freon-114 methods alone, the modified procedure yields a product with only trace amounts of total solids, lipids, and proteins; with increased specific infectivity; and with no loss in biological activity.

A simple procedure for obtaining the organism of psittacosis relatively free of lipids and proteins contributed by chicken embryo yolk sac, the host tissue, has been reported previously.\* This procedure employed calcium chloride, sodium dextran sulfate, and sucrose (CC-DS-S) with a single low-speed centrifugation step to give a high quality, partially purified product.

Yolk sac suspensions of the organism of psittacosis have also been extracted with the fluorocarbon dichlorotetrafluoroethane,\*\* without any biological inactivation;\*\*\* however, a single extraction removed only 30% of the protein and 15% of the lipid.

Because both of the above procedures are easy to perform, but neither reduced the extraneous solids, protein, and lipid content to trace amounts, it occurred to us that an adaptation of both procedures might result in a simple procedure that would give a product of higher purity. Chicken embryo yolk sac material was partially purified as described by Shirey et al.

\* Shirey, William N.; Dorsey, Warren G.; Patrick, William C., III. 1965. A practical method for partial purification of the organism of psittacosis grown in chicken embryo yolk sac. Biotechnol. Bioeng. 7:447-454.

\*\* Freon-114, E. I. Du Pont de Nemours and Company, Wilmington, Del.  
\*\*\* Comer, J.F.; Wachter, R.F. 1963. Effect of dichlorotetrafluoroethane on the infectivity of viruses and rickettsiae sensitive to trichlorotrifluoroethane. Bacteriol. Proc. V61:144. (Abstr.)

(CC-DS-S procedure). The resulting product was treated with Freon-114 as follows: Reagents, culture, and equipment were cooled to -3 to +3 C. One part Freon-114 was mixed with two parts of the CC-DS-S product in a Waring Blender operated at 10,000 rpm for 30 seconds. The resulting suspension was centrifuged for 10 minutes at 700 x g in a refrigerated centrifuge at -3 C. The clear upper aqueous layer was decanted and tested for biological activity by mouse inoculation. The number of mouse ICLD<sub>50</sub> (MICLD<sub>50</sub>) in relation to the level of lipids, proteins, and total solids was used as a criterion for judging purity. The characteristics of the slurry of the psittacosis organism after each successive treatment are shown in Table 1. An examination of these data indicates a marked improvement in purity of the yolk sac suspension of psittacosis organisms when partial purification by the CC-DS-S procedure was followed by Freon extraction.

The total solids content was markedly reduced, as were the lipid and protein contents, with each purification step. Only trace quantities remained after application of the Freon-114 extraction procedure. The specific infectivity index (MICLD<sub>50</sub> per gram of protein) increased significantly with each purification step. A percentage computation that reflects the degree of improvement in purity of psittacosis product suspensions is shown in Table 2. The Freon-114 extraction procedure resulted in no loss in biological activity but reduced the solids content 387-fold and the total protein content 300-fold and increased the specific infectivity factor 560-fold. A partially purified product was obtained with a volume of only one-fifth of the original volume of crude material and with less than 1% of the extraneous yolk sac components.

TABLE I. THE EFFECT OF THE CC-DS-S AND THE FREON-114 PROCEDURES IN THE PURIFICATION OF 50% YOLK SAC SUSPENSIONS OF THE ORGANISM OF PSITTACOSIS

Sample	Volume, ml	Titer, MICLD <sup>a</sup> / ml, $\times 10^8$	Specifics/ Infectivity MICLD <sub>50</sub> $\times 10^{12}$	Solids, mg/ml, $\times 10^2$	Lipids, mg/ml, $\times 10^1$	Protein, mg/ml, $\times 10^1$
50% Yolk sac	540	3.60	0.012	1.40	74.00	30.30
CC-DS-S Product	100	17.40	1.230	0.90	1.33	1.41
Freon-114 Product	100	37.00	6.727	0.02	0.33	0.55

a. MICLD<sub>50</sub> per gram of protein.

TABLE 2. RECOVERY OF INDICATED FACTORS AFTER THE CC-DS-S  
PROCEDURE AND THE FREON-114 PROCEDURE

Sample	Volume, %	Titer, %	Solids, %	Lipids, %	Proteins, %
50% Yolk sac	100	100	100	100	100
CC-DS-S Product	19	90	12	0.3	0.9
Freon-114 Product	19	160+	0.3	0.08	0.3

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION	
Department of the Army Fort Detrick, Frederick, Maryland, 21701		Unclassified	
2b. GROUP			
3. REPORT TITLE			
AN IMPROVED METHOD FOR PARTIAL PURIFICATION OF THE ORGANISM OF PSITTACOSIS GROWN IN CHICKEN EMBRYO YOLK SAC			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)			
5. AUTHORITY (First name, middle initial, last name) Dorsey, Warren G. Shirey, William N. Patrick, William C., III			
6. REPORT DATE	August 1967	7a. TOTAL NO. OF PAGES	7b. NO. OF REPS
8. CONTRACT OR GRANT NO.		9a. ORIGINATOR'S REPORT NUMBER(S)	
10. PROJECT NO. 1B533001D426		Technical Manuscript 408	
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY	
		Department of the Army Fort Detrick, Frederick, Maryland, 21701	
13. ABSTRACT			
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14. Key Words			
Psittacosis Yolk sac Purification Freon Centrifugation Fluorocarbon			

DD FORM 1 MAY 64 1473

REPLACES DD FORM 1473, 1 JUN 64, WHICH IS  
COMPLETE FOR ARMY USE.

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